

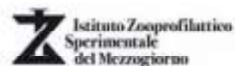
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EVALUATION OF DIFFERENT PCR PROTOCOLS FOR DETECTING MAEDI VISNA VIRUS IN SHEEP WITH HISTOLOGICAL LESIONS AND POSITIVE IMMUNOHISTOCHEMICAL RESULTS IN NORTHERN SPAIN

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Maedi-Visna (MV) is a widespread disease responsible for direct losses in sheep production. MV is characterised by a slow but progressive infection in sheep, resulting in a chronic interstitial inflammation of lungs, mammary glands and joints, and in a non-suppurative encephalitis and demyelination of the central nervous system (CNS). Prevalence in Spanish Assaf dairy sheep ranges between 44% and 96% among flocks and >80% in half of them (1). Maedi Visna Virus (MVV) is characterised by a high genetic variability, which may affect the sensitivity of diagnostic tests. The aim of this work was to evaluate different PCR protocols for detecting MVV in samples from Spanish Assaf sheep with histological lesions and resulted positive by immunohistochemistry (IHC).

Archival frozen samples from 6 sheep and formalin-fixed and paraffin embedded (FFPE) samples from further 8 sheep were available for PCR. The animals were Spanish Assaf sheep submitted to the Pathology Diagnostic Service. CNS, lung, or udder showed lesions referable to MV, p28 antigen of MVV was detected by IHC, and all but 2 animals were positive by nested PCR (2). DNA obtained from frozen or FFPE samples was tested by different PCR protocols (3, 4, 5, 6, 7, 8). On the basis of preliminary results, new primers were designed and used in single or in nested PCRs in combination with other primers (2) to amplify a sequence of the LTR gene. PCR products were sequenced and compared with known sequences (6).

The best results were obtained when the products of PCR based on external primers previously described (2) were used in nPCR with the new primers (100% sensitivity). The sequences (about 800 bp) obtained by the gag-pol PCR (6) from the 2 animals positive only using the new primers showed higher identity with sequences previously found in Spanish sheep (9), although identity was lower than 90%. No products were obtained by gag-pol PCR on DNA from FFPE samples, probably because DNA degradation.

Further studies are required for obtaining more genetic sequences from samples with discordant PCR results and for studying the phylogenetic relationship among different MVV strains.

[1] Minguijón E et al. *Vet Microbiol*, 181: 75-89, 2015. [2] Ryan S et al. *J Virol*, 74(21): 10096-103, 2000. [3] Rosati S et al. *J Vet Diagn Invest*, 7: 437-443, 1995. [4] Reina R et al. *Virus Research* 121: 189-198, 2006. [5] Shah C et al. *Virology* 319 (1): 12-26. [6] Grego E et al. *J. Gen. Virol.* 88: 3423-3427, 2007. [7] Eltahir YM et al. *J Virol Methods*, 135(2): 240-246, 2006. [8] Grego E et al. *Clin. Diagn. Lab. Immunol.* 9 (4):828-832, 2002. [9] de Andrés X et al. *Vet Immunol Immunopathol.* 15;152(3-4):277-88, 2013.